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**Running title:** Reverse causation in epidemiologic studies of PFOA

## Abstract

**Background:** Impaired kidney function and earlier menopause were associated with

perfluorooctanoic acid (PFOA) serum levels in prior cross-sectional studies. Reverse causation,

whereby health outcomes increase serum PFOA, may underlie these associations.

**Objective:** We compared measured (subject to reverse causation) vs. modeled (unaffected by

reverse causation) serum PFOA in association with these outcomes, to examine the possible role

of reverse causation in these associations.

**Methods:** In cross-sectional analyses, we analyzed PFOA in relation to self-reported menopause

among women (N=9,192) aged 30-65, and kidney function among adults aged 20+ (N=29,499)

in a highly-exposed Mid-Ohio Valley cohort. Estimated glomerular filtration rate (eGFR, a

marker of kidney function) and serum PFOA concentration were measured in blood samples

collected during 2005/06. Retrospective year-specific serum PFOA estimates were modeled

independently of measured PFOA, based on residential history and plant emissions. Using

measured and modeled PFOA in 2005/06 (predictor variables), cross-sectional associations were

assessed for eGFR and menopause (yes/no). We also analyzed measured PFOA (dependent

variable) in relation to the number of years since menopause.

**Results:** Menopause and eGFR were significantly associated with measured (trend tests:

p=0.013, p=0.0005, respectively) but not modeled serum PFOA (p=0.50, p=0.76, respectively).

Measured PFOA levels increased for the first seven years after menopause (trend test, p<0.0001),

providing further evidence that the observed association between measured PFOA and

menopause is subject to reverse causation for this outcome.

**Conclusion:** Our results support the conjecture that in prior studies, early menopause and reduced kidney function are the causes rather than the results of increased measured serum PFOA. These results suggest caution in using biomarkers in cross-sectional studies.

## Introduction

Though serum biomarkers of chemical exposure are often seen as objective measures of internal dose, their use in cross-sectional studies introduces the possibility of reverse causation, since measurement of both disease and the biomarker are concurrent. Causal relationships between chemical exposure and disease may be particularly questionable if the outcome of interest can impact a chemical's pharmacokinetics by, for example, decreasing excretion and consequently increasing accumulation in the body.

While reverse causation is a well-known concern in cross-sectional studies of outcomes and measured biomarkers including biomarkers of chemical exposure, demonstration of this problem is not common. Demonstration of reverse causation is facilitated by an alternative exposure measure, invulnerable to possible reverse causation, which can be used instead of the potentially affected exposure biomarker; often such an alternative measure is not available. In the present work, we use measured PFOA levels in the blood and an alternative measure, modeled PFOA levels based on estimates of external exposure. We show two specific examples of likely altered excretion rates of perfluorooctanoic acid (PFOA) resulting from two health outcomes, menopause and renal function, and discuss the potential impact of reverse causation on epidemiologic analyses of each outcome in cross-sectional analyses.

PFOA, a perfluoroalkyl acid, was widely-used in the manufacture of polymers, such as Teflon. PFOA is found in serum at low levels of almost all of the U.S. population ( median=4 μg/L; Calafat et al. 2007), is not metabolized (Post et al. 2012), and is slowly excreted, with a reported half-life ranging from 2.3-3.8 years (Bartell et al. 2010; Olsen et al. 2007).

At menopause, menstruation, which accounts for a yearly blood loss of 10-14% of blood volume (see Supplemental Material), ceases. Without this mode of recurring excretion in chronically exposed women, rates of PFOA accumulation in blood after menopause may be greater than before menopause. Decreased serum PFOA has been shown in pre-menopausal versus post-menopausal women (Harada et al. 2005), and analogously, in men experiencing regular blood withdrawals in the course of medical treatment (Lorber et al. 2015). Two cross-sectional studies, one of women exposed at background PFOA levels (Taylor et al. 2014) , and one of women in a large, highly-exposed Mid-Ohio Valley cohort (Knox et al. 2011), found positive associations between higher measured serum concentrations and earlier menopause. In exploring possible reverse causation, Taylor et al. also found a positive linear association ($\beta$= 0.07, 95%CI: 0.013-0.13) between the number of years after natural menopause and the log-transformed measured serum PFOA concentration (2014), suggesting that decreased excretion after menopause led to higher serum levels. Pharmacokinetic modeling studies at background exposure levels have noted that accounting for menstruation partially explained observed higher serum perfluoroalkyl acids (including PFOA) concentrations in men versus women (Lorber et al. 2015; Wong et al. 2014) or in girls who are not yet menstruating versus those who are (Wu et al. 2015).

Analogously, lower renal function (calculated as estimated glomerular filtration rate (eGFR)) may result in decreased PFOA excretion and a consequent increased rate of accumulation in serum. Cross-sectional analyses of adults exposed at background levels (Shankar et al. 2011) and children exposed at high levels (Watkins et al. 2013) found a positive association between lower kidney function (i.e., lower eGFR) and higher

measured serum PFOA. Watkins et al. were unable to confirm this relationship when using an environmentally modeled exposure estimate of serum PFOA instead of a measure in blood serum.

In separate work, we have analyzed longitudinally the relationship between modeled PFOA and both diagnosed chronic kidney disease (CKD, characterized by severely diminished renal function) and menopause, (Dhingra et al. 2016a, 2016b). In neither case did we find evidence of association. In this context, we consider the possibility of reverse causation in the afore-mentioned prior cross-sectional associations of measured serum PFOA.

To explore reverse causation in associations of both kidney function and menopause with PFOA, we compare measured and modeled serum PFOA cross-sectionally at the time of blood draw (2005-06), as predictors of eGFR and menopause prevalence. If PFOA exposure truly affects either outcome of interest and the association is observed with measured PFOA, then we expect the association to also be observed when using modeled serum PFOA in place of measured (assuming the modeled metric is reasonably accurate). If PFOA does not alter kidney function (or analogously menopause prevalence), measured PFOA might still appear inversely associated with eGFR (or positively associated with menopause) if pre-existing decreased renal function (or analogously ceased menstruation) impacts PFOA excretion and hence serum levels.

We also examined yearly accumulation of serum PFOA after menopause. Due to the loss of excretion via menstrual blood, we expect that post-menopausal women would on average have increasing serum PFOA for  several years after menopause as compared

to premenopausal women; this would also suggest that reverse causation might explain

prior observed cross-sectional associations of measured PFOA and earlier menopause.

## Methods

Our Mid-Ohio Valley community is centered around DuPont manufacturing

facilities near Parkersburg, WV, where PFOA releases began in 1951 (Frisbee et al.

2009). This community was exposed to PFOA higher than background exposures

(median measured serum concentration was 28.2 μg/L in 2005/06; Frisbee et al. 2009).

### Data: Surveys, PFOA and eGFR

The present cohort (N=30,303) consists of Mid-Ohio Valley residents who were

studied cross-sectionally in the C8 Health Project (C8HP) and longitudinally by the C8

Science Panel (C8SP). Figure S1 shows cohort formation. The present cohort consists of

C8HP participants aged at least 20 years who provided informed consent to participate in

subsequent C8SP studies, which includes the present study. The Institutional Review

Board at Emory University reviewed and approved all aspects of these studies, including

consent forms and surveys.  The current study is covered under that IRB approval.

Our study population is the same Mid-Ohio Valley community studied cross-

sectionally by Watkins et al. (2013) for eGFR, cross-sectionally by Knox et al. for

menopause (2011), and longitudinally by Dhingra et al. (2016a, 2016b) for CKD and

menopause. To be included in the C8HP, conducted in 2005/06, individuals must have

been exposed for at least one year to PFOA-contaminated water from an affected water

district or a contaminated private well (Winquist et al. 2013). The C8HP included a blood

sample for measurement of serum PFOA concentrations (μg/L) and other biomarkers,

including creatinine (Frisbee et al. 2009). To calculate eGFR in adults, creatinine levels

were entered into the Modification of Diet and Renal Disease study equations, a validated

method for determining eGFR in Caucasian and African American adults (NKDEP

2014).

In C8SP surveys (2008-11), participants provided information on demographics,

health behaviors (e.g., smoking, exercise), and their history of several chronic diseases.

Residential history (1951-2011), including dates at each residence, was collected for each

participant. Women were asked about their reproductive history, including menstrual and

pregnancy history (Winquist et al. 2013). Serum PFOA concentrations were modeled for

each year as part of the C8SP studies (Winquist et al. 2013).

Methods for generating year-specific (1951-2011) estimated PFOA serum

concentrations were previously described in detail (Shin et al. 2011a, 2011b; Winquist et

al. 2013; Woskie et al. 2012) and summarized in the Supplemental Material. The

modeled exposure estimates were generated through a set of models with inputs based on

environmental information and some information collected from participants, including

residential history, body weight, work history and drinking water source and

consumption rate (when provided).  It is unlikely that either of the outcomes considered

here would influence reporting of these factors. Concurrent (i.e., modeled in the year of

blood sampling) serum PFOA estimates have a Spearman correlation with serum PFOA

concentrations measured in the blood sample (2005/06) of 0.71 (Winquist et al. 2013).

All analyses used modeled or measured estimates of serum PFOA determined in 2005-

06, the time of blood draw, and included only cohort members with both measures.

## Analyses: eGFR and PFOA in adults

Of our cohort's 30,303 adults, 590 were excluded because they were born before 1920 (consistent with prior C8SP analyses), were missing covariates, were missing serum creatinine for eGFR determination, or were younger than 20 in 2005/06. An additional 72 people with implausibly high eGFR (>150) were excluded, leaving 29,641 subjects for analysis. Using linear regression, eGFR was regressed in separate models on three PFOA exposure metrics: measured serum PFOA concentrations; modeled serum PFOA concentrations; and modeled cumulative exposure (the sum of all year-specific, modeled serum concentrations up to a given year), while adjusting for the potential confounders (see Table 1 footnote). As Shankar et al. (2011) adjusted for high cholesterol and hypertension presumably as potential confounders, we ran the above model additionally controlling for hypertension and high cholesterol (both self-reported as physician diagnosed) as a sensitivity analysis.

Each exposure variable was categorized into quintiles in the primary analysis, while the log-transformed continuous PFOA metric served as a trend test. To further clarify the dose-response relationship between measured serum PFOA and eGFR, measured serum PFOA was additionally categorized into deciles. Regression coefficients and p-values for modeled and measured PFOA were compared to assess the potential for reverse causation.

## Analyses: Menopause and PFOA

Of 16,870 women in the present cohort, we excluded 4,455 who were less than 30 or greater than 65 years of age. We excluded an additional 3,223, who had incomplete menopausal history (N=517), reported menopause before age 30 (N=1,589), or were

missing covariates. Of the remaining 9,192 women, 2,355 had experienced natural menopause and 2,850 had experienced hysterectomy, while the remainder had experienced neither.

*Cross-sectional logistic regression.* Following prior cross-sectional analyses by Knox et al. (2011), we conducted logistic regression in which reported natural menopause as of 2005/06 was the outcome. For this analysis, we further restricted the cohort to women aged 40-60 in 2005/06 to ensure that every age had both post-menopausal and pre-menopausal women, and excluded women with hysterectomies, following Knox et al. (2011); 6,342 women remained after these exclusions. Exposure variables were constructed as in the eGFR analysis, using both measured and modeled exposure.

*PFOA and years since menopause.* In the full cohort of 9,192 women, cross-sectional linear regression analyses were conducted with log-transformed measured serum PFOA as the outcome variable and the number of years elapsed since menopause at the time of blood draw in 2005/06 (hereafter, referred to as 'years since menopause') as the main predictor. Women with hysterectomies were retained in this analysis and hysterectomy was considered as equivalent to menopause, in leading to no more PFOA excretion via menstruation. We used either a linear variable or a 2-year categorical variable for 'years since menopause.' The referent group ('years since menopause' = 0) comprised pre-menopausal women and those reporting that menopause occurred in the year of their blood sample collection (newly menopausal). To capture any non-linear relationship between 'years since menopause' and log-transformed measured serum levels, we used a restricted cubic spline with three knots (Harrell et al. 1988) and a linear spline with one knot. Knots were chosen to maximize fit, judged via $R^2$.

In addition to adjusting for known predictors of measured serum PFOA (Steenland et al. 2009a), we also included, as a covariate, modeled serum PFOA in 2005/06, which adds predictive value to the model because it accounts for other factors not included in the other predictors, such as detailed residential history over time. As some known predictors are potentially related to modeled serum PFOA, we alternately removed either 'known predictors' or modeled serum PFOA from the model as part of a sensitivity analyses. Both 'known predictors' and 'modeled PFOA' independently contributed to predicting measured PFOA, as judged by the model R-square.

## Results

### eGFR analyses

Table S1 shows cohort characteristics for the eGFR analyses. There was a negative trend in eGFR across measured serum PFOA quintiles (Table 1; $\beta$=-0.64, -1.03, -0.84, -0.98 for 2nd-5th quintiles vs. 1st quintile). Log-transformed continuous measured serum PFOA (a test for trend) was negatively associated with eGFR (p=0.013). Neither modeled serum PFOA nor modeled cumulative exposure showed association with eGFR (p=0.43 and p=0.66, respectively). The sensitivity analysis including hypertension and high cholesterol, showed very similar results to our primary analysis. Examination of eGFR and deciles of measured serum PFOA gave a dose-response curve that decreased until the 4th decile and remained approximately flat thereafter (Figure 1a).

### Menopause analyses

Table S2 shows cohort characteristics for the menopause analyses. We found a significant increasing trend of reported menopause with increasing measured PFOA category (trend test: p = 0.04; Table 1), after adjustment for age and other potential

confounders, similar to the findings of Knox et al. (2011). When using modeled serum or

modeled cumulative exposure PFOA instead of measured, this trend disappeared (p=0.90

and p=0.48, respectively). Decile categorization of measured serum PFOA as a predictor

of menopause showed a dose-response curve that increased up to the 4th decile and then,

with the exception of a drop at the 5th decile, remained approximately level thereafter

(Figure 1b).

All regression models of log-transformed measured serum PFOA in relation to

'years since menopause,' showed positive, significant associations between more 'years

since menopause' and measured serum PFOA (Figure 2). Although all exposure metrics

for 'years since menopause' had similar fit as judged by R-square, varying slightly

around 0.68 among metrics, the two-piece linear spline model appeared to best follow the

2-year categorization of years since menopause. The two-piece linear spline showed a

steady increase of approximately 4% per year in measured PFOA with each additional

year since menopause until year seven, after which no further increase was observed.

Alternative sensitivity analysis models that excluded either 'known predictors' or

modeled serum PFOA produced associations varying only slightly from our main model.

## Discussion

Our analyses furnish evidence that reverse causation led to associations in cross-

sectional analyses of associations between PFOA and both renal function and earlier

menopause. Measured serum PFOA concentrations showed a significant negative

association with eGFR, but neither modeled serum PFOA nor modeled cumulative

exposure showed this association. The negative association between eGFR and measured

PFOA is consistent with an increase in serum PFOA that might be expected to result from decreased kidney clearance of PFOA.

Similarly, cross-sectional logistic regression models of menopause showed that measured serum PFOA was positively associated with increased risk of menopause, while modeled exposure metrics were not. Measured serum PFOA was on average 4% higher per year for the first seven years after menopause, as compared to pre-menopausal and newly menopausal women, after controlling for other factors known to influence serum PFOA levels in this cohort. This result is consistent with the cessation of PFOA excretion via this route after menopause proposed by Harada et al. (2007). After 7 post-menopausal years, this increased rate of accumulation appears to cease in our data, perhaps resulting from the establishment of a new steady state between intake and excretion, or more error in the reported menopausal age further from the event (Rodstrom et al. 2005).

Causal diagrams of the modeled relationship between the available PFOA metrics and the outcomes are presented in Supplemental Material (Figure S2). These diagrams present both modeled and measured serum PFOA as imperfect measures of what might be the true etiologically relevant PFOA exposure for either menopause or eGFR, were there to exist a causal association between PFOA and our outcomes. When postulated in our causal diagrams this way, 'reverse causation' be thought of as measurement error of the exposure which differs by outcome status, as discussed in Hernan and Cole (see Figure 2c in Miguel A. Hernan 2009).

Were a true causal relationship between POFA and menopause to exist, neither measured nor modeled PFOA in 2005/06 could be the etiologically relevant exposure,

which often occurred years earlier, before the time of menopause. However, among

menopausal women, serum PFOA modeled at time of menopause occurrence (i.e., closer

in time to the potentially relevant exposure) is highly correlated with serum PFOA

modeled in 2005/06 (Spearman R=0.86) and reasonably correlated with measured serum

PFOA (Spearman R=0.64) in 2005/06. If there was a causal relationship from PFOA to

menopause, these observed correlations suggest that modeled PFOA would be more

strongly associated with reported menopause (yes/no) in 2005/06 than would measured

PFOA. Our finding that measured exposure has a positive relationship with earlier

menopause, while the modeled does not, can be considered evidence of reverse

causation.  An analogous argument could be made for eGFR, regarding the critical time

of exposure if a causal relationship existed, although unlike menopause specific time of

changes in eGFR are not known.

In these analyses, we believe that observed associations of each outcome with

measured serum PFOA but not with modeled serum PFOA provides evidence that the

outcome may impact the measured exposure metric. However, in the presence of a true

causal relationship from PFOA to either outcome, an inaccurate model of serum PFOA

exposure could also yield null results for modeled exposure (e.g., non-differential

mismeasurement error), as in our findings. Still, we believe our modeled PFOA is

reasonably accurate. We found a good correlation between modeled PFOA in 2005/06

and measured PFOA in that same year (R=0.71), suggesting that the model performs

well.  A previous study that used the same modeled exposure estimates used in our study

assessed  the impact of exposure measurement error on the validity of epidemiologic

models for preeclampsia  (Avanasi et al. 2016). While these authors showed that

uncertainty in measurements of source data, such as observed water concentrations,

might impact the serum PFOA estimates themselves, they had little effect on the rank

order of these estimates or on effect estimates for preeclampsia.

Furthermore, the modeled serum PFOA estimates that were used in the present

analyses, have allowed detection of other epidemiologic associations that are in

agreement with other studies. They have been used for example to show an association

between PFOA and high cholesterol (Winquist and Steenland 2014), which was

previously demonstrated in cross-sectional analyses using measured serum PFOA

(Steenland et al. 2009b) in the same cohort; this association is now well-established in the

literature (e.g., Costa et al. 2009; Frisbee et al. 2011). The modeled exposure also

predicted testicular cancer in a longitudinal study in our population, an outcome, which

was predicted  priori based on animal data (Barry et al. 2013).

It should be noted that modeled estimates may also be subject to reverse causation

if self-reported values of some variables in the model were influenced by health outcome

status. One example might be past self-reported water consumption. Higher self-reported

water consumption might lead to over-estimates of modeled serum PFOA.  However,

both of our outcomes are not generally thought of by subjects as 'disease' (low GFR is

generally asymptomatic, and subjects would not consider menopause as a disease).

Consequently, those subjects with lower kidney function or menopause are not expected

to over-report their water consumption rate as a result of the subject's perception that

high water consumption led to their 'disease'.

On the other hand, our outcomes could be also associated with true water

consumption. For example, there is some evidence in the literature that those with low

GFR might consume less water (e.g., Clark et al. 2011; Sontrop et al. 2013). As a result, those with disease would report less than expected water consumption rates, thus biasing modeled PFOA serum levels downward among those with lower eGFR. This could in turn bias any true causal association between modeled PFOA and GFR to the null. While we cannot exclude this possibility, the phenomenon would also result in a downward bias in measured PFOA among those with lower eGFR, which is contrary to our observation that those with lower GFR have higher measured PFOA. Water consumption is the only reasonably plausible variable used in modeling PFOA which might be affected by low eGFR, and was but one variable among many affecting modeled PFOA. More importantly, water consumption is only a weak predictor of modeled PFOA and thus unlikely to have any important impact. In a regression of modeled PFOA on water consumption, age, gender, and current water district, the total model $R^2$ is 0.41, and the partial $R^2$ for water consumption is only 0.03.  In contrast, the partial $R^2$ for water district is 0.34. Residential history is a much more important variable than water consumption for determining modeled PFOA, and it is unlikely to be related to eGFR level. Regarding menopause, we can find no data in the literature associating menopause with changed water consumption.

Non-differential misclassification of outcomes could also result in biases to the null.  For example, substantial intra-individual variation in creatinine clearance and thus in measured eGFR (Levey et al. 2015) may bias results of PFOA and eGFR analyses toward the null, assuming outcome mismeasurement did not differ by exposure level. This error could impact analyses of both measured and modeled PFOA. Self-reports of menopausal age are subject to recall bias (Rodstrom et al. 2005) and possible

digit preference (Crawford et al. 2002). If participants are, at interview, much older than their menopausal age, this may introduce measurement error into menopausal status (yes/no) and estimates of years since menopause, though we do not expect exposure status to impact recall of menopausal age. Such error might particularly affect our analyses of measured PFOA and 'years since menopause' (which included women who had been post-menopausal for many years), resulting in more error at the highest levels of years since menopause.

Despite the above-noted limitations, in our analysis it appears that elevated measured serum PFOA concentrations resulting from reduced excretion can lead to misleading cross-sectional analysis results. Nonetheless, those elevated concentrations do reflect true elevated internal dose, which may have implications for other health outcomes.

## Conclusions

Internal measures of dose may not be appropriate for some cross-sectional epidemiological studies due to reverse causation. Often biomarkers of exposure are preferable when models yield only imprecise estimates of external environmental exposure. For outcomes that may impact the biomarker level, however, cross-sectional results should be interpreted with caution. If a reasonably accurate modeled estimate of internal exposure (dose), derived without the opportunity for distortion by individual variation in pharmacokinetics, is available, it may be preferable in cross-sectional epidemiological studies.

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## Tables

*Table 1. Results of cross-sectional regression of eGFR and PFOA (N=29,641) and logistic regression of PFOA and Menopause (N=6,342).*

| PFOA Exposure variable | eGFR analysis, linear regression [c] | | Menopause analysis, logistic regression [d] | |
| --- | --- | --- | --- | --- |
| | Parameter estimate±S.E | p-value | OR (95%CI) | p-value |
| **Measured serum concentration (2005/06), quintiles (ref. = 1st quintile)[a]** | | | | |
| 2nd | -0.64± 0.268 | 0.018 | 1.68 (1.21, 2.35) | 0.002 |
| 3rd | -1.03±0.269 | 0.0001 | 1.45 (1.04, 2.02) | 0.03 |
| 4th | -0.84±0.271 | 0.0019 | 1.39 (1.00, 1.93) | 0.05 |
| 5th | -0.98±0.274 | 0.0003 | 1.58 (1.14, 2.19) | 0.006 |
| **Measured serum concentration (2005/06), log-transformed[e]** | -0.14±0.07 | 0.03 | 1.09 (1.002, 1.18) | 0.04 |
| **Modeled serum concentration (2005/06), quintiles (ref. = 1st quintile)[b]** | | | | |
| 2nd | -0.08±0.268 | 0.77 | 0.98 (0.70, 1.37) | 0.90 |
| 3rd | 0.37±0.268 | 0.17 | 1.05 (0.75, 1.45) | 0.78 |
| 4th | 0.21±0.269 | 0.44 | 0.78 (0.56, 1.08) | 0.14 |
| 5th | 0.23±0.271 | 0.41 | 0.92 (0.65, 1.30) | 0.62 |
| **Modeled serum exposure (2005/06), log-transformed[e]** | 0.05±0.058 | 0.43 | 0.98 (0.70, 1.37) | 0.90 |

OR= Odds ratio, CI= confidence intervals, S.E.= standard error

[a] In eGFR and menopause analyses, upper cutpoints of 1st, 2nd, 3rd and 4th quintiles are 11.1, 19.4, 36.3, & 88.0 μg/mL, and 9.7, 17.2, 31.9, & 78.8 μg/mL, respectively.

[b] In eGFR and menopause analyses, upper cutpoints of 1st, 2nd, 3rd and 4th quintiles are 5.8, 11.4, 26.8, & 82.4 μg/mL, and 6.1, 11.8, 26.8, & 78.0 μg/mL, respectively.

[c] Potential confounders were chosen, a priori, from the literature, and included smoking status (current/former/never), body mass index (BMI) (<18.5, 18.5-25, 25-30, ≥30 kg/m2), education level ('less than high school (HS),' 'HS diploma,' 'some undergraduate education,' 'bachelor's degree or higher'), race (white vs. non-white), gender, and birth year (Anderson et al. 2009; Haroun et al. 2003; Saydah et al. 2007).

[d] Based on a review of factors associated with menopause (Gold 2011), covariates in the model included age in 2005/06 (linear term); parous/nulli-parous status; smoking status; education; BMI in 2005/06; and birth year (linear or 2-yr categories).

[e] Serum concentrations were log-transformed using log base e.

## Figure legends

**Figure 1.** Dose response curves (a) showing changes in eGFR (mL/min/1.73m$^2$) with decile of measured serum PFOA, (b) showing the odds ratios for natural menopause by decile of measured serum PFOA. The first decile is the reference and error bars designate the 95% confidence interval.

**Figure 2.** Percent increase in measured serum PFOA as a function of 'Years since menopause. Covariates included known predictors of serum PFOA measured in 2005/06: smoking status; education; BMI; growing one's own vegetables; high cholesterol; diabetes; current residential water district; having previously lived/worked in a contaminated water district; bottled water consumption; well water consumption; birth year (2-yr. categorical variable); evidence of having worked at the DuPont plant; month of blood sample collection (categorical variables representing two month intervals); and modeled serum PFOA in 2005/06.
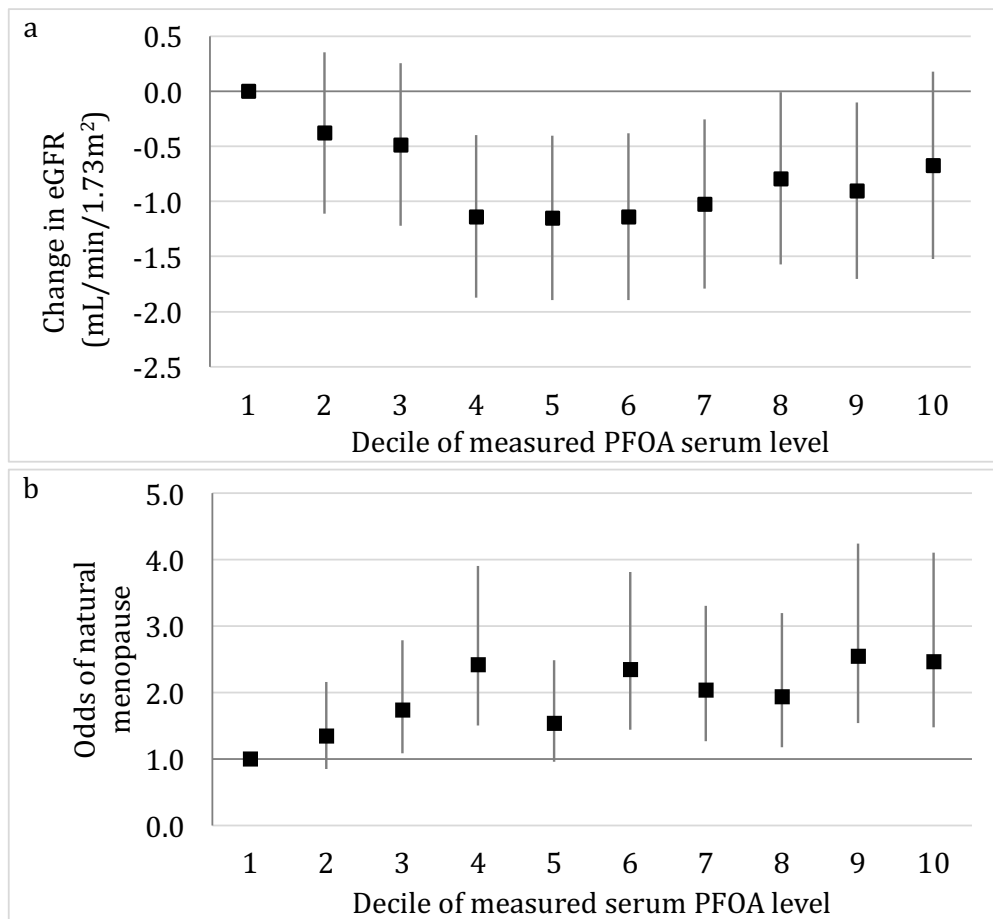
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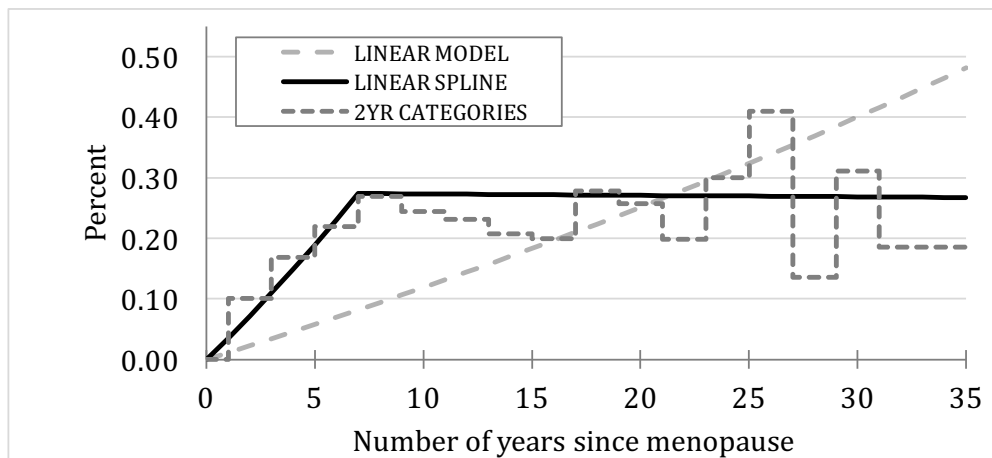
Figure 2.



*Figure 2. Percent increase in measured serum PFOA as a function of 'Years since menopause. Covariates included known predictors of serum PFOA measured in 2005/06: smoking status; education; BMI; growing one's own vegetables; high cholesterol; diabetes; current residential water district; having previously lived/worked in a contaminated water district; bottled water consumption; well water consumption; birth year (2-yr. categorical variable); evidence of having worked at the DuPont plant; month of blood sample collection (categorical variables representing two month intervals); and modeled serum PFOA in 2005/06.*